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An Address

ON

BACTERIOPHAGY AND RECOVERY FROM INFECTIOUS DISEASES*

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WE know through common experience that certain species of animals are completely refractory to certain diseases which decimate other species. No one has ever seen, for example, in the course of the most terrible epidemic, a single rabbit contract cholera or a single guinea pig contract typhoid, although men were dying by thousands. The guinea pig and the rabbit, as indeed all other animals, are refractory to these two diseases. *They enjoy a natural immunity.*

We know, likewise, by common experience, that a great many of the infectious diseases do not recur or, at least, recur but rarely. It is unusual for a man who has recovered from an attack of typhoid, for example, to contract this disease a second time. A first attack of an immunizing disease leads, therefore, within the individual, to the appearance of a new character. *He enjoys an acquired immunity.* This immunity, very strong at the beginning, gradually diminishes at a rate more or less rapid in accordance with the disease causing it. In certain cases it disappears completely after a greater or less length of time.

There is, in addition, a third type of immunity. In certain of the chronic diseases, such as tuberculosis or syphilis, it is very evident that the patient does not enjoy an acquired immunity, since the pathogenic organisms continue to develop within the lesions, but he possesses, nevertheless, a new character, for reinfection can not occur so long as he remains the carrier of the specific germs. This immunity, certainly different from acquired immunity, since it ceases at the moment when the specific organism disappears from the lesion, may be termed pathogenic immunity or, better, *symbiotic immunity.*

It is only natural *a priori* to consider the phenomenon of recovery as being within the limits of immunity, but this has yet to be experimentally proved. It is somewhat curious to note that this question of recovery in infectious diseases, a question which would seem fundamental, has always been passed over in silence. Everyone has implicitly admitted that recovery was a natural consequence of the acquisition of immunity. The reason for this conclusion can readily be understood for all present day immunology is founded upon laboratory experiments, carried out with guinea pigs and rabbits. These animals have been inoculated with cultures of different bacteria, cholera vibrios, typhoid bacilli and others for which they possess an absolute natural resistance. In them have been produced artificial infections which bear no relationship with natural diseases. It is in this way that nature has been disobeyed, for such studies can only lead to an imaginary solution. Let us illustrate the fact by an example.

Cole and Dochez have found that an adequate amount of anti-pneumococcus serum, type 1, is able to save the life of a mouse previously injected with a million fatal doses of type 1 pneumococcus

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culture, thus the serum is highly valuable. Well, experimenting with the same serum, Cole treated 431 cases of type pneumonia with a death rate of 10.2; no control series was studied. Locke had a death rate of 17 amongst the treated and exactly the same death rate amongst the controls. In Wadsworth's series the death rates amongst treated and control were respectively 18 and 19 per cent. My conclusion is that the serum under experiment has been very successful in the artificial pneumonia in mice, and worthless in the natural pneumonia of man. In reviewing the literature I could quote hundreds of examples emphasizing the fact that artificial infections induced in laboratory animals bear no relationship with natural disease in man. For ten years I have fought to emphasize this fact, without any success. The same pseudo-experimental method continues and will continue to be current practice in the study of immunology.

In so far as recovery is concerned, a simple observation of the facts suggests that it cannot be the consequence of an acquired immunity. As a matter of fact all of the infectious diseases, not to mention the pyogenic infections, are not immunizing; it is certain that bacillary dysentery can recur at frequent intervals, and that the relapses are often as serious, if not more serious, than the initial attack. As for cholera, it is not rare to see recurrences, and all authors of the nineteenth century who have seen epidemics in Europe have been unanimous in considering cholera as a non-immunizing disease. On the other hand, in the case of those diseases which are actually immunizing, if recovery results from the acquisition of immunity, how are we to explain satisfactorily the relapses which take place during convalescence, at the moment when the immunity should be at its maximum potency? Take for example the case of typhoid fever. Relapses are not uncommon; they occur during convalescence, generally within twenty days of the abatement of the symptoms, and, far from being benign, they are severe and often fatal. Well, at the moment of the relapse the antibodies are present at their highest potency. This proves that antibodies do not provoke the recovery, and, furthermore, that immunity was not yet established at the moment of the recovery from the first attack. These observations suggest the hypothesis that recovery can take place without the phenomena of immunity intervening and that acquired immunity, far from being the cause of the recovery, must appear from ten to twenty days after the recovery. In a word immunity must be not the *cause* but the *consequence* of the recovery.

In order to verify this hypothesis, suggested by observation, it is necessary to study what takes place within the patient at the moment of recovery, and to do this by adopting an experimental method which conforms to the principle of obeying nature. It is necessary to study the man or the animal afflicted with natural disease, to see the phenomena which take place within him at the time of recovery and throughout the course of convalescence. This method is the only one which can provide a true solution to the problem. From the beginning of my study of bacteriophagy I have been struck by the fact that the appearance within the body of the patient of the principle which leads to bacteriophagy coincides with the time when the symptoms ameliorate. Absent during the disease, bacteriophage appears constantly in convalescents. *Bacteriophagy is thus contemporaneous with recovery.*

In vitro, bacteriophagy consists of the following. Let us take a few drops of stool derived, for example, from a convalescent from bacillary dysentery, Let us emulsify this in about 20 c.c. of sterile bouillon and filter it through a porous porcelain filter, such as the Chamberland, or through a silica candle, such as the Berkefeld. Let us add to a young bouillon culture of the dysentery bacillus a drop of this filtrate, and place the tube in the incubator. At first the bouillon appears cloudy, but after a few hours we note that it becomes clearer and clearer, and, finally, after about 12 hours, sometimes more quickly, it becomes perfectly limpid. At this time all of the bacilli are dissolved.

Let us take, next, a new, fresh culture of dysentery bacilli and add to it a drop of the limpid fluid which remains after the disappearance of the bacilli from the first mixture. Let us place this second tube in the incubator. We will find that the phenomenon repeats itself, for after a few hours all of the bacilli are again dissolved and the liquid is clear. We may then remove a drop of the second dissolved culture and introduce it into a third culture of dysentery bacilli. Once more the phenomenon of dissolution takes place. One might in this manner continue the passages indefinitely, introducing into each new, fresh culture of dysentery bacilli a drop of the preceding one after all of the bacilli have been dissolved. Far from diminishing in intensity

in proportion to the degree of dilution of the initial drop of filtrate the phenomenon becomes, on the contrary, more intense. Thus it is that after more than a thousand successive passages I have been able to obtain the complete dissolution of the 2,000 million bacilli contained in 10 c.c. of bouillon by adding the infinitesimal quantity of a billionth of a cubic centimetre of the preceding dissolved culture.

Such experiments demonstrate that the principle which destroys the bacteria, and to which I have given the name "Bacteriophage," reproduces itself in the course of its action. The phenomenon of bacteriophagy consists essentially, then, in a dissolution of bacteria under the influence of a principle which reproduces, the latter phenomenon, that is, reproduction, being directly related to the bacteria which are dissolved.

Various questions now arise. Is this bacteriophage found only by chance in the intestinal tract of certain dysentery patients, or is it a constant occurrence? We will return to this question later. Is the phenomenon of bacteriophagy limited to the dysentery bacillus? I have been able to establish the fact that bacteriophagy is a general phenomenon. It has been possible to isolate races of bacteriophage leading to the dissolution of bacteria belonging to very varied species, such as *Eberthella dysenteriæ, paradysenteriæ, typhi, paratyphi,* and *sanguinaria; Escherichia coli; Salmonella schotmülleri, pullora, suipestifer,* and *typhi-murium; Proteus vulgaris; Vibrio comma; Pasteurella pestis* and *bovis; Corynebacterium diphtheriæ,* and *B. subtilis.* Other investigators have isolated bacteriophage races active with staphylococci, streptococci, and pneumococci, and even with bacteria parasitic in plants, such as *Rhizobium radicicolum, B. tumefaciens,* and *B. carotovorus.* The diversity of the bacteria attacked warrants the belief that the phenomenon is, indeed, general, perhaps involving all bacteria.

Various experiments show that the bacteriophage exists in corpuscular form. One such experiment, the most simple, consists in adding to 10 c.c. of a well-grown bacterial culture an infinitesimal trace, a millionth of a cubic centimetre, of a filtrate containing active bacteriophage. If a drop of this culture is spread immediately on agar we will obtain, after incubation, a growth of bacterial culture over the surface of the media, and this will be spotted with circular bare areas which appear to be perfectly sterile and are visible to the naked eye. Each of these plaques represents a colony of bacteriophage made up of millions of corpuscles, all the issue of a single corpuscle deposited on the agar at the time of spreading. Each corpuscle has commenced to multiply at the expense of the neighbouring bacteria. The destruction of the bacteria and the simultaneous multiplication of bacteriophage corpuscles is so active that after a few hours the area of destruction is so wide as to be visible to the unaided eye.

For lack of time I will not discuss all of the chacteristics of the bacteriophage phenomenon for it is in reality extremely complex. I will restrict myself to some of the essential ideas. The bacteriophage corpuscle is a living, ultramicroscopic being, as is proved by the fact that this corpuscle dissolves bacteria through the agency of a ferment which it secretes. The secretion of a ferment implies a metabolism and this is an essential character of living beings. A bacteriophage is, therefore, of necessity a *virus,* a *parasite* of bacteria.

In its action each bacteriophage is not specific, for a given bacteriophage may parasitize and dissolve bacteria belonging to different species, sometimes as unrelated as the streptococcus and the colon bacillus or even the plague bacillus and *B. typhosus.* The characters of each strain of bacteriophage are variable. There are races of bacteriophage able to attack many species of bacteria, others which attack but a single species or even but a single bacterial strain. Certain of them are so potent that they are able *in vitro* to destroy and to dissolve within less than two hours all of the bacteria contained in a culture, while others exercise but a scarcely perceptible, partial action.

Adaptability is an exclusive property of living beings and the bacteriophage possesses this character to a very high degree. There are, however, in this respect differences between different races, for certain bacteriophages adapt themselves very readily, while others do so very slowly. In so far as the present discussion is concerned, the most important character of adaptability is represented by the faculty which each strain of bacteriophage possesses of adapting itself to the parasitism of new bacterial species which heretofore were not attacked. This experiment of adaptation can even be effected *in vitro.* It is possible, for example, to adapt a bacteriophage which originally, at the time of isolation, was active only upon *B. coli* to the

parasitism of *B. typhosus*. This property of adaptation is rapidly lost in races of bacteriophage maintained under laboratory conditions.

There is another important consideration. When attacked by a powerful bacteriophage the bacterium succumbs, but if the potency of the bacteriophage is less the bacterium is capable of resistance, in which case it then contracts a true chronic disease accompanied by profound modifications in its characters. With regard to the subject now under discussion the most important of these modifications involves the variation in bacterial virulence, and this is usually attenuated and may completely disappear. As we shall see later, this phenomenon of variation of the virulence of bacteria resisting the action of bacteriophage is very important in relation to the cause of infectious diseases. The resisting bacteria live for an indefinite number of generations in symbiosis with bacteriophage, that is to say they suffer from a chronic disease caused by bacteriophage, for all the modern researches on symbiosis (which is a very general phenomenon in nature) lead to the conclusion that symbiosis is always a chronic disease in which the resistance of the host balances the virulence of the parasite. The symbiosis continues as long as the two antagonistic powers are perfectly balanced; if the virulence of the parasite, or the resistance of the host increase, symbiosis is broken in favour of the former or the latter.

In brief, these are the principal characters of the very complex phenomenon of bacteriophagy.

We have seen that the principle which causes bacteriophagy can be uniformly isolated from the convalescent, and we are able to conclude that bacteriophagy *in vivo* is contemporaneous with recovery. Is it the cause? In the first place let us state that the bacteriophage virus does not appear spontaneously at the moment of recovery. Experiment demonstrates that the bacteriophage exists in the intestinal content of all healthy individuals, where it grows at the expense of the saprophytic bacteria, of *B. coli* in particular, which are daily ingested with the food. Thanks to its faculty of adaptation, the normal intestinal bacteriophage becomes able to parasitize foreign bacteria which may become implanted, not only within the intestines but in any organ whatsoever, for experiment shows that the bacteriophage passes readily into the circulation. In order to prove if the bacteriophage is really the cause of a recovery it is only necessary to study patients affected with acute infectious diseases from the beginning of the attack up until the end of convalescence. This is what I have done for various human and animal diseases. Here is, in short, what I hav observed. The condition of the patient depends upon the behaviour of the bacteriophage and recovery takes place only when the destroying potency of the bacteriophage reaches an intensity sufficient to lead to the bacteriophagy of the pathogenic bacteria.

I am not able, for lack of time, to describe the many studies which I have made upon this subject during different epidemics in Europe, in Indo-China, and in India. Here are, as an example, the results of the studies made upon cholera. These results are expressed in the form of curves,* with the solid line representing the severity of the disease as determined by the different symptoms. Ten represents the maximum severity where all of the symptoms are present to the highest degree, 0 indicates the absence of symptoms, that is, recovery. The dotted line presents the curve of potency of the bacteriophage isolated from the patient at the same time in its action upon the cholera vibrio. Between 10 and 6 on the scale, tests of potency *in vitro* show that there is a complete bacteriophagy in a time varying from 2½ hours (10) to 12 hours (6); below 6 the bacteriophagy is only partial, becoming less as the coefficient drops: 0 represents no activity upon the cholera vibrio. As may be seen here from these curves, not only is the phenomenon of recovery strictly related to the behaviour of the bacteriophage, but the condition of the patient at a given moment is always a function of the activity of the bacteriophage. If bacteriophagy does not take place the patient dies. In bacillary dysentery, in typhoid and the paratyphoid fevers, in different animal septicemias, and in human and murine plague the examination of patients reveals the same relationship between recovery and bacteriophagy *in vivo*.

It is necessary, however, to emphasize the fact that the phenomenon of bacteriophagy *in vivo* is far more complex than that occurring *in vitro*. In the latter case only two beings are involved, the bacterium and its parasite, the bacteriophage. *In vivo* a third factor enters—the host. In my first book on bacteriophage, published in 1921, I described experiments which tend to show

* This refers to lantern slides displayed at the lecture.

that opsonins are in reality the lysins secreted by the bacteriophage corpuscles during bacteriophagy. Let us take two tubes containing like mixtures of sensitive bacteria and leukocytes, and let us add to one of these tubes a suspension of bacteriophage or a solution of lysin freed from bacteriophage corpuscles. We will find that phagocytosis is from five to fifty times more active in the presence of the lysin that it is in the control tube. The opsonic power of bacteriophage has since been confirmed by various investigators, including Gohs and Jacobsohn, Weiss and Arnold, Nelson, and Smith.

This opsonic action tends naturally to prevent the formation of secondary cultures which are so frequent *in vitro*, but, even though these may be formed, a third phenomenon intervenes which also depends entirely upon the bacteriophage. I have stated above that bacteria which resist the action of bacteriophage undergo modifications in their characters and principally in that of their virulence, which generally becomes attenuated and often disappears completely. This it is easy to prove experimentally. Three distinct phenomena, therefore, take place *in vivo*—bacteriophagy itself, a powerful opsonic action, and an attenuation in the virulence of the pathogenic bacteria. These three are induced by bacteriophage and all contribute toward recovery.

But recovery or death are not the only two possible issues. There is a third one, the passage to a chronic state in which the symptoms are more or less apparent, sometimes even lacking, as is the case in "carriers", which are to be considered as two chronic patients. We know, for example, that in the typhoid carriers, the gall-bladder is infected. The passage to chronicity is due to the fact that in the body, as well as *in vitro*, bacteria are able to resist the bacteriophage and to form a symbiosis. In such a symbiosis the characteristics of the bacteria are transformed, as I have stated before. In the case of certain bacteria, as cholera vibrio, the virulence is utterly destroyed, with the result that cholera carriers are absolutely harmless; in the case of other bacteria, such as typhoid bacilli, the virulence of the symbiosis is variable. I have recently performed experiments with the bacterium of the same group, *Salmonella typhi murium*, a very convenient bacterium, for it is possible to provoke in mice a natural disease by oral administration of a minute quantity of culture. I have obtained in the test tube a whole series of "mutations" of the salmonella, under the action of bacteriophage, each of these "mutations" is a "symbiosis". I have tested the virulence of eight of these: two are completely avirulent; two have a virulence about the half of the primitive pure salmonella; four have a very low virulence and provoke, not an acute disease, as is the case with the primitive salmonella, but a disease lasting several weeks.

For lack of time, I have not the opportunity to discuss the relationship between acute and chronic diseases from one part of the behaviour to the symbiosis bacteria-bacteriophage, from another part, anyhow, the experiments with *Salmonella typhi murium* are sufficient to show that such relationship exists. In brief, acute diseases are causes by "ultrapure" bacteria, chronic diseases are caused by a symbiosis formed between bacteria and bacteriophage.

All these studies tend, therefore, to show that recovery is in no way derived from a phenomenon of immunity as had been believed up to the present time, but rather that it is a direct result of bacteriophagy *in vivo*. It is, furthermore, easy to prove this conclusion, for it may be done by means of crossed experiments. It is possible, easy indeed, to introduce into a culture of the pathogenic bacterium a trace of a suspension of a virulent bacteriophage; the bacteria are attacked and destroyed, and meanwhile the bacteriophage multiplies. That which was at the beginning a culture of bacteria becomes, after a few hours, a culture of bacteriophage. Let us administer to a patient, at the onset of symptoms, a few drops of this culture of bacteriophage. Bacteriophagy must take place *in vivo* and recovery must follow. The patient will not be forced to take the chance of his own intestinal bacteriophage undergoing an adaptation, for we can inaugurate, at the beginning of the disease, the natural processes of recovery.

I will state briefly what has been done up to the present time in this direction. From 1919 onward I have made experiments upon patients affected with bacillary dysentery, causing each patient to ingest two cubic centimetres of a culture of bacteriophage having a high virulence for dysentery bacilli. In all cases, without exception, all of the morbid symptoms disappeared within a few hours, in from four to twenty according to the case, and the next day the patient was definitely convalescent. Since that time this method of treatment has been applied on a large scale, principally in the Soudan and in Brazil.

In Brazil, as the result of control experiments conducted by da Costa Cruz, who obtained results identical with those I had reported, the Oswaldo Cruz Institute of the Brazilian Government has prepared, since 1924, cultures of a virulent bacteriophage for the dysentery bacilli. These have been placed into two cubic centimetre ampoules and distributed to hospitals, to government health officers, and to all physicians who have requested them. This mode of treatment has quickly supplanted all others, including the use of antidysenteric serum, which has been abandoned. The results obtained in the first 10,000 cases have been published and only two failures are recorded.

As for the Soudan, this phrase, summarizing the results, appears in a letter of the Director of the Public Health Service. "The results of treatment of bacillary dysentery with it have been little short of miraculous." A single failure, the case of an infant already moribund when brought into the hospital, occurred among several hundred cases treated.

I must admit that several experimenters have not obtained the same favourable results. As we shall see later, the power of the phage utilized for the treatment is an essential factor of success. It is not sufficient to administer any bacteriophage in order to obtain recovery; the *sine qua non* of success is to administer a *powerful* bacteriophage. It seems that many authors have not yet realized that this condition is imperative. As an example, I have quoted the paper of Riding, who experimented in the Soudan, and has reported a complete failure. He states that he utilized a bacteriophage which was furnished to him by myself. This statement is misleading, for at the time indicated (autumn of 1927), I could not send any bacteriophage from Egypt, as after the month of March I was in India experimenting with cholera. I suppose that the bacteriophage received by Riding had been prepared by the bacteriologist from my service in Egypt during my absence and I do not know what was its value. But what is still more surprising is that Riding alluded in his paper to the letter summarizing the results obtained the year before with bacteriophage, which I had really prepared, and treated it as an "absurd statement", but without indicating, who made the statement and where the experiment had been performed. Readers would have been certainly cautious about the failure of Riding if he had had the honesty to say that the experiment had been performed the preceding year in Khartoum, and that the "absurd statement" had been written by the Director of the Medical Service of the Soudan.

In the year 1927, while in India, as the result of the experiments of which I have spoken, I attempted the treatment of Asiatic cholera. These attempts were made in the Punjab, on natives cared for in their homes and to whom no other medication was given. Each patient received an initial dose of two cubic centimetres of a virulent bacteriophage, and with the family a second dose of four cubic centimetres diluted in one hundred cubic centimetres of water was left with instructions to give it to the patient by spoonfuls during the three or four hours following. I should explain that I merely furnished the cultures of bacteriophage. Treatment was carried out by Major Malone, of the Indian Medical Service, assisted by the other officers of the Service. As it was impossible to enforce any one mode of treatment, the family of the patient was free to accept or refuse it, in the latter case usually resorting to the prescriptions of the Hindoo medicine man. The majority of the patients for whom authorization was granted were found in a critical state; indeed, it was only because of this that parents, despairing of saving them, accepted the new treatment. As a control series we have taken those cases in which the bacteriophage treatment was refused. In spite of these extremely unfavourable conditions the mortality in the controls was 62.9 per cent, and among those treated with bacteriophage, 8.1 per cent. Since then Colonel Morison of the Indian Medical Service has applied the same mode of treatment in epidemics of cholera in Assam, and, working also in the villages, he has obtained comparable results, the mortality varying from 8 to 11 per cent among the treated, while the mortality among those not treated by bacteriophage varied from 60 to more than 80 per cent according to the epidemic. Asheshov has treated patients in the hospital by applying bacteriophage treatment by the intravenous route and he has succeeded in lowering the mortality to about 3 per cent.

Two experimenters, Tyler in Assam, and Souchard in Indo-China, have recorded a complete failure, but it appears from their own texts that the strains of the bacteriophage they have utilized had been in cultivation in the laboratory for a long time and were of no potency. I have always emphasized that any attempt of

treatment with such a phage would lead to complete failure.

Let us pass to another disease which has a high mortality, which has also furnished the most striking results. In 1926, while in Egypt, I treated four cases of bubonic plague, injecting the bacteriophage into the buboes; all four of the patients recovered. In the course of an epidemic which occurred last year in Senegal, Dr. Couvy, Director of the School of Medicine at Dakar, faced with the non-effectiveness of antiplague serum in severe cases of the disease, attempted the treatment by bacteriophage, utilizing a strain isolated from a convalescent. In order to ascertain in a definite manner the value of this treatment he applied it solely to cases of extreme severity, in whom death seemed to be certain within a short time. "Either they appeared moribund after failure of the serum treatment, or it was given at once to patients whose condition appeared desperate," as he stated in his paper. Among such patients the mortality is practically one hundred per cent, but with bacteriophage treatment he obtained 15 recoveries among 21 cases treated. In the course of this epidemic 8 cases of septicæmic plague were treated by serum before the trial of bacteriophage. All of these died. Two cases treated by bacteriophage recovered, in spite of the fact that the bacilli were so abundant in the blood that they could be disclosed by direct microscopic examination. Of nine cases of pneumonic plague treated by serum all died (as is well known, pneumonic plague is without exception fatal,) while one case treated with bacteriophage recovered. "The action of bacteriophage", states Couvy, "manifests itself by an abrupt fall in the temperature; often the defervescence is violent, a fall of several degrees. The general condition rapidly improves. The antitoxic action is most sharp and the hallucinations quickly give place to calm. The periadenitis disappears, the buboes retrogress, and convalescence takes place within a few days. One never sees the interminable suppurations so frequent with other methods of treatment. There is no necrosis or gangrene."

Let us state in passing that the antitoxic action manifested so quickly and effectively by the bacteriophage is absolutely clear cut, although it is difficult to explain in the present state of our knowledge. I have observed it not only in plague but in other toxic diseases which I have treated with bacteriophage, cholera and bacillary dysentery among others. Gerard, in Madagascar, has tried the administration of bacteriophage in three cases of primitive pneumonic plague. Two patients recovered, the condition of the third showed marked improvement, but he died from a relapse. Anyhow, these results are very promising, for the prognosis in primitive pneumonic plague is fatal, without any exception.

Let us pass on to other diseases having a high mortality. We know that recovery is rare in staphylococcus septicæmia, the mortality being about 99 per cent. In 1929, at my suggestion, Dr. Davioud treated a hopeless case in the following manner. Five cubic centimetres of a suspension of staphylococcus bacteriophage was diluted in 500 c.c. of physiological saline. This was all introduced intravenously, the period of injection occupying about one hour. This is, indeed, the technique which I have recommended for all intravenous injections of bacteriophage, and it is possible to inject in this manner without danger of immediate shock as much as 25 c.c. of a suspension of bacteriophage. Two hours after the injection a marked pyrexia occurred with chills. Upon the morning of the next day the temperature was normal and convalescence began. Eight days later the patient left the hospital, recovered. When seen ten months later she had enjoyed perfect health. Since then several other cases of staphylococcus septicæmia have been treated in the same manner in the hospitals of Paris with a like degree of success.

Dutton, the first, I believe, who has treated with success cases of streptococcus septicæmia, and Raiga, has very recently treated this condition. Since here defervescence did not take place as quickly after the injection as in the preceding cases, upon my advice he made a series of ten intramuscular injections of 5 c.c. each, with an interval of 24 hours between each injection. Here is an example. A woman of 28 years, with a puerperal infection was treated simultaneously with septicæmine, pyoformine, immuno-transfusion, fixation abscesses, and anti-streptococcus serum. On the third of July, confronted with the failure of all these methods and by the fact that the patient was gravely ill, the physician in charge requested Dr. Raiga to apply bacteriophage. The temperature was then 41° C. He gave an intravenous injection of 10 c.c. of streptobacteriophage diluted in 500 c.c. of physiological saline. No reaction followed. In spite of the fact that the temperature continued during the following day to vary between 40 and

41°, and the blood cultures remained positive, the condition of the patient improved and the appetite returned. Since two injections of anti-streptococcus serum had been made in the two flanks there had developed in these regions a diffuse phlegmon. On the right the infiltration had progressed toward the anterior region of the thigh and had assumed the appearance of a gangrenous phlegmon. Purulent fistulæ extended to the knee. On the 18th of July a series of 10 daily intramuscular injections of 5 c.c. of streptococcus bacteriophage were commenced. On the 23rd of July the slough was removed. Upon the 31st of July the blood culture became negative and the temperature progressively lower. The patient left the hospital cured on the 31st of August.

Let us turn to still another type of disease—typhoid fever. Since 1923 many papers have appeared upon the subject of its treatment by bacteriophage. Some of the authors (Hauduroy, Alessandrini, and Doria among others) reported excellent results, while with others (Wolff, for example) the results were negative. I believe, however, that I have recently discovered the cause of these differences. I will speak of them shortly when I consider the general conditions governing treatment by bacteriophage.

For two years I have studied this question and have made experiments in many centres in France. One experiment, involving about 150 cases, shows that if one administers by mouth a suspension of bacteriophage (I have used a mixture containing many strains of typhoid bacteriophage and several strains of coli bacteriophage) in a dose of 2 c.c. repeated every 4 hours, one does not obtain a cure in the strict sense of the word, but the disease develops in the form of a simple fever without complications. The stools are formed and normal, the patient does not complain of any disturbance, and regains his appetite. None of these cases have died. On the other hand, when one applies bacteriophage by the intravenous route, in the manner indicated above, one induces in about half of the cases a strong thermic reaction with chills followed by a rapid fall in the temperature, which reaches normal in 48 hours. In the other half of the cases no reaction takes place and the disease follows its normal course. In view of these results, and it is in this direction that I intend to continue my studies, it would seem that an intravenous injection of typhoid bacteriophage might be given, continuing the treatment in the cases where the salutary reaction does not take place, either by administration by mouth, as in the first series of cases mentioned, or by serial intramuscular injections, such as those used in the streptococcus septicæmias.

In infantile diarrhœas I have applied treatment by bacteriophage in several hundred cases, using a mixture containing a large number of races of bacteriophage active upon the different pathogenic bacilli which may be found in the intestines of patients,—dysentery bacilli of the Flexner or Hiss types, Morgan bacilli, and *B. proteus*. Bacteriophage is administered by mouth in doses of 2 c.c., this being repeated every two hours until the stools have become normal. The effect is usually very prompt and in more than 80 per cent of the cases recovery is obtained within twenty-four hours. The results would certainly be still much more favourable if new strains of bacteriophage were added, for it seems that infantile diarrhœa is not a definite entity from the standpoint of etiology but may be caused by bacteria of various types. By using a mixture containing bacteriophage capable of acting upon all of those bacteria which cause infantile diarrhœa one might hope to be successful in 100 per cent of cases. This appears the more probable, since I have found that the efficiency of the treatment increases as new races of bacteriophage are added to the preparation used for the treatment of this disease.

I will only mention here the treatment of urinary infections due to colon bacilli, for a great many authors have studied this problem and have published their results. It may be said that in acute infections prompt recovery is the rule following intravesicular injections of coli bacteriophage active for the colon bacillus causing the disease. It must also be said that recovery is the rule in these infections whatever the treatment employed. In chronic cases a review of the results indicates that about 60 per cent of the cases recover when treated by instillation into the bladder in conjunction with a series of subcutaneous or intramuscular injections. This figure is raised to about 85 per cent if the instillations are made, not into the bladder but into the pelvis of the kidney involved. It should be added that it has not yet been possible to isolate strains of bacteriophage acting upon all the cultures of coli which may be found in chronic cases. In a given case of chronic infection caused by this organism it is first

essential to determine whether the bacillus of the patient is attacked by a stock bacteriophage. If it is not, it is essential to utilize an "auto-bacteriophage". This difficulty is not present in other diseases, for with the exception of *B. coli* races, we now possess strains of bacteriophage which are polyvalent.

Bacteriophage treatment of staphylococcus infections has been very extensively applied. Since the general principle of the method consists in placing the bacteriophage in as intimate a contact as is possible with the pathogenic bacterium it is essential in staphylococcus infections to inject directly into the focus. Since such injections may often be very painful one may, as Jaquemaire has shown, mix with the staphylo-bacteriophage a quantity of a suitable anæsthetic, novacaine for example. This treatment has, up to the present, been applied in thousands of cases. For example, Raiga alone has reported the results of this treatment on more than 1,000 different cases, Thurman Rice, 300 cases. The series of Larkum is very large, and Halphen has used the treatment in 600 cases of tonsillar abscesses, etc. Other types of staphylococcus infection have been treated with equal success, such as furunculosis, carbuncles, paronychia, abscesses of all kinds, of the gums, of the breast, and rectal abscesses. It has been used in phlegmons, in infected wounds, and in osteomyelitis. All investigators who have used this mode of treatment in these different infections are unanimous in stating that the results obtained are far superior to those secured with other methods. This is especially true as regards the rapidity of the action and the absence of scars, which, of course, are very significant in connection with lesions of the face. One interesting fact recorded by several authors, which I also have observed upon several occasions, is that very quickly after the first injection of bacteriophage into the pyogenic focus the pain disappears completely. The patient who, prior to the intervention, was continually moaning, after one or two hours experienced a sensation of euphoria. This action is especially striking in the case of certain very painful abscesses, those involving the anal region for example. This is not a specific action in particular patients but is a general effect.

One other type of infection should be mentioned briefly, that is, the treatment of chronic bronchitis, of angina, and of coryza by means of a mixture of different races of bacteriophage active for those organisms which may be isolated from the throat in these conditions. The bacteriophage is here applied by spraying the nose and throat. I have seen the results in about 300 cases of these different conditions treated in this way and in from 60 to 70 per cent recovery was rapid. As is the case for other diseases in which the specific germ varies, the results would certainly be improved with the addition of new strains of bacteriophage to the stock.

I must end this lecture in which, for lack of time, I have been forced merely to cite facts without entering into the details of each particular case, with a few general considerations.

Treatment by bacteriophage has been, I believe, demonstrated to be the specific treatment *par excellence*, since it leads to recovery through a mechanism identical with that of natural recovery. Because of its nature one may hope to obtain results only when the bacteriophage administered is endowed with a maximum potency against the pathogenic organism involved. As we have seen, it is possible to isolate very powerful races of bacteriophage; other races are less active and may be very weak indeed. Any attempt at treatment with any type of bacteriophage of low potency is to court a certain defeat. The *sine qua non* of success is the utilization of bacteriophage races selected with care.

I would add a second statement, which is equally important. I have recently discovered that the therapeutic effect of a bacteriophage is the stronger the more recently the bacteriophage has been isolated. After a series of cultures in the laboratory, although *in vitro* the virulence of the bacteriophage is maintained intact, it loses more or less quickly its power of acting *in vivo*. Preliminary studies already indicate that this attenuation of *in vivo* action is due to the fact that gradually, as cultivation continues, the bacteriophage loses the faculty of adaptation. In plague, for example, the attenuation is so marked that after four or five laboratory passages the bacteriophage has lost all therapeutic action. Nevertheless, this same race, tested *in vitro*, shows no weakening in its ability to attack plague bacilli. The same facts have been noted in cholera, and are probably true also for typhoid fever. With the staphylococcus bacteriophage, on the contrary, it would seem that the therapeutic action may be maintained for a very long time through passages *in vitro*. This attenuation of action *in vivo* is caused by the passages *in vitro*,

and is not due to the period of preservation, for a bacteriophage which has undergone but two or three of these passages and is then preserved in sealed ampoules retains intact its properties for many months or even for several years.

A third observation may be made. Whatever the disease under consideration the bacteriophage must be administered in such a way that it can quickly come into contact within the body with the bacteria which it is designed to destroy. This condition can always be readily met, since one has only to select the mode of administration suited to each particular case. The question of posology is of no very great importance, since the bacteriophage commences to multiply just as soon as it comes into contact with susceptible bacteria. Theoretically, a billionth of a drop should suffice, provided the bacteriophage corpuscles are placed in contact with the bacteria which they are to destroy. *In vitro* this fact is readily demonstrated, but it is not the same *in vivo*. However, since the bacteriophage has no action upon the cells of the body, and since, as a result of this, it is possible to administer an unlimited quantity without inconvenience to the patient, even if the diagnosis is erroneous, and since it is always desirable to induce a rapid destruction of the pathogenic bacteria, it is in general wise to administer reasonably large doses. In relation to this subject I might state that as a result of laboratory experiments which have been poorly interpreted I had recommended that intramuscular or subcutaneous injections be not repeated. Subsequent experiments have shown that such a statement was not justified, and that it is often possible to administer a series of ten or fifteen large doses, that is 5 to 10 c.c.

If I may now make a final recommendation, I would say that bacteriophage destined to therapeutic usage should be prepared in accordance with a proved technique. It should not contain bacteria and it is, indeed, easy to demonstrate whether this condition is fulfilled. Suspensions of bacteriophage are perfectly clear, despite the fact that billions of bacteriophage corpuscles are present. The slightest turbidity in an ampoule indicates a certain contamination and such material should not be used.

Bacteriophage therapy is still in its infancy and many studies are still necessary before we shall demonstrate all the results that we may anticipate, but what has already been done in many diseases justifies the belief that this is the specific treatment *par excellence* and that it will attain a wider and wider application.

THE UNCERTAIN POTENCY OF LIVER EXTRACTS IN THE TREATMENT OF PERNICIOUS ANÆMIA*

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DURING the past two years it has become more and more apparent that certain preparations of liver extract used for the treatment of pernicious anæmia are inadequate to control the disease. It is not the purpose of this communication to expose any one brand of extract to the advantage of others, but rather to draw attention to certain facts which are well known to those who have facilities for making assays of the substance and for following closely a considerable number of cases treated by liver extract. It is my aim to present evidence for the consideration of those who prescribe the extract indiscriminately, and also for those who are responsible for its manufacture. One of the leading manufacturers in the United States at one time suspended its production for a considerable period, placing it again on the market only after being reasonably certain of its potency. It would seem that other manufacturing pharmacists might well follow their example.

IMPORTANCE OF EARLY RECOGNITION AND ADEQUATE TREATMENT

The consequences of failure to recognize pernicious anæmia in its incipiency and to treat it adequately are often of incalculable importance to the victim of the disease. It is now generally recognized that signs and symp-

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